

Diversity and Functional Characterization of Endophytic Bacteria from *Crassula Ovata* with Emphasis on Plant Growth Promotion and Antimicrobial Activity

Vibhuti Rana¹ and Monika Singh^{1*}

¹Department of Biotechnology, School of Applied and Life Sciences, Uttarakhand University, Dehradun, Uttarakhand, India-248007

*Correspondence: monikasingh_bhu@yahoo.com

Received: 14th June, 2026; Revised: 20th July 2026; Accepted: 16th September, 2026; Available Online: 20th September 2026

ABSTRACT

Endophytes can serve as a reservoir for a range of secondary metabolites with biological activity, particularly those that are extracted from herbal plants. *Crassula ovata* belongs to genus *Crassula* and family *Crassulaceae*. It is a succulent and evergreen plant with thick branches. This study was aimed to isolate endophytic bacteria from leaves, stems, of *Crassula ovata*. Nineteen endophytic bacteria were isolated, among the all, five pure endophytic bacteria were taken for biochemical characterization and plant growth activity. These strains were selected on the basis of their excellent growth. All the strains showed positive result for IAA, urease production and most of them exhibited phosphate solubilisation, ammonia and nitrate reduction tests. Catalase, motility, starch hydrolysis and H₂S production some strains showed partially positive results. Out of five, only two bacterial endophytes were identified with 16SrRNA gene sequencing. The aim of this study, to identify any endophytic bacteria with the PGP activity to support the sustainable agriculture in extremely dry or desert conditions.

Keywords: *Crassula ovata*, endophytes, Scanning electron microscopy, biochemical activity, plant growth promoting activity, antibiotics sensitivity; 16 S rRNA

How to cite this article: Rana V, Singh M, Diversity and Functional Characterization of Endophytic Bacteria from *Crassula Ovata* with Emphasis on Plant Growth Promotion and Antimicrobial Activity.

Int J Drug Deliv Technol. 2026;16(83s):1700-1710. DOI: 10.25258/ijddt.16.83s.185

Source of support: Nil.

Conflict of interest: None

1. INTRODUCTION

Endophytic microorganisms residing internal plant tissues without causing any harm (Stone *et al.*, 2000). Every part of the plant, such as fruits, shoots, and roots, has endophytic microbes (Sturz *et al.*, 2000). Endophytic bacteria have multiple positive impacts on the host plant (Brooks *et al.*, 1994; Berg *et al.*, 2005; Tan *et al.*, 2006; Rijavec *et al.*, 2007). Many microorganisms commonly coexist with plants, some of which may promote the development or activation of plant defence mechanisms. Rhizobacteria, epiphytic, and endophytic bacteria are all related to plants. Without appearing to harm their host, endophytic bacteria infiltrate the inside tissue of the plants. The studies of potential beneficial effects on crop production in the last few years have sparked interest in beneficial endophytic bacteria. Furthermore, a great deal of research has demonstrated that endophytic bacteria can promote plant development or function as biocontrol agents against plant diseases. Biological control activities via producing antibiotics, siderophores, specific enzymes, nutrient competition, the creation of complete resistance, increased mineral element availability, and decreased manufacture of ethylene are just a few of the effects that endophytic bacteria have on plants. Although endophytic bacteria can colonise aerial sections of plants, much

experiment has concentrated on endophytic bacteria of root, and small is known about the variety of prokaryotic germs found in host tissues like leaves or stems. Discovering strains that help forest trees grow more quickly may have positive consequences and enhance environmental quality. *Acinetobacter*, *Pseudomonas*, *Sphingomonas*, *Actinobacteria*, *Bacillus* and species in the family *Enterobacteriaceae* are the most frequently isolated types of endophytic bacteria from forest trees (Oshnoei *et al.*, 2017). In ancient times plants have been used as medicines. *Crassula ovata* is a decorative plant and also a remedial plant. It used as home remedy for treatment of diarrhea, disinfecting wounds, to healing wounds and it has potential to control stomach upset. It contains alkaloids, saponin, steroids, sterols, and active carbohydrates. These are chemical elements that are actively involved in reducing microbial activity. Now-days most people take medicinal plant product on day today life to improve their good health as much as to treat illness (Muiruri and Mwangi., 2016). *Crassula ovata* plant is also known as Jade plant a medicinal herb that has been used for centuries to treat various infections and diabetes related illnesses also. Yet, only intermittent attempts have been undertaken to scientifically and methodically validate these conventional claims up to this point. South Africa is the

*Author for Correspondence: monikasingh_bhu@yahoo.com

original home of the plant *Crassula ovata*. It is also a common indoor plant all over the world, however it is mostly found in the Northern Hemisphere, particularly in places that are cold, dry, and have little access to water. The plant can endure droughts, being grazed on, trampled on, or knocked over thanks to its succulent water-storing leaves, stems, and roots. It can also root from any part of its stem, even just a single leaf (Chokhone *et al.*, 2017). According to a recent study of the plant *Crassula ovata*, pure cultures of the isolates were sub-cultured on various media to evaluate the characteristics of macro conidia, micro conidia, and *chlamydospores*. Komada's *Fusarium*-selective medium was used to isolate the fungal cause of the illnesses. Pure cultures were kept at 7°C after the isolates were sub-cultured on PDA (potato dextrose agar). Growing the fungi on the media was described, along with the morphological characteristics of the colonies of each isolate, macro conidia, micro conidia, and *chlamydospores*. On *Covanta*, *Echeveria agavoides*, and *Papavernudicalule*, *Fusarium oxysporum* isolated and reported for the first time in Italy and around the world (Ortu *et al.*, 2014). The current study examines the biological activities and phytochemical content of 40 species from nine genera in the family *Crassulaceae*. Many traditional medical systems have traditionally employed plants to treat sickness. The globe is currently dealing with new alignments that require the use of more cutting-edge medications. A sustainable supply of lead compounds with a potential for effectiveness in the treatment of various illnesses can be found by examining the phytochemical composition of plants. In Africa, the leaves are used to treat diarrhoea, epilepsy, and corns by boiling them in milk. In India, leaf juice is used to cure diabetes. Antimicrobial antioxidant, and antidiabetic activities of *C. ovata* has been documented. Extracts of the *C. ovata* stems and leaves in aqueous and methanol are effective against *E. coli*. The plant contained sterols, sugars, phenols, alkaloids, and flavonoids, saponin, according to a phytochemical examination. According to chemical diversity, the family *Crassulaceae* a range of secondary metabolites, with sterols, phenolic triterpenes, and diterpenes being the most prevalent (Hassan *et al.*, 2021). The extraction of various medications and chemotherapeutics from medicinal plants as well as from a number of conventionally used herbal treatments has led to an increase in the reliance on their use in industrialised cultures. Despite the fact that medicinal plants are being used more frequently, a lack of care for their protection is endangering their future. Because of rising trade demands for less expensive healthcare products and new plant based therapeutic markets instead of more expensive biopharmaceuticals and target specific drugs, herbal and medicinal plant reserves in developing countries are depleting and in danger of extinction (Ahmed *et al.*, 2014). It has been demonstrated that introducing plant growth-promoting rhizobacteria (PGPR) into plants is an efficient way to reduce the stress that drought and other environmental conditions generate in maize plants (Pereira *et al.*, 2020). When PGPR were co-inoculated, the aerial

biomass of plants subjected to a moderate water deficit increased by up to 89%. Additionally, bio-inoculation improved the utilization of phosphorus (P) and nitrogen (N), which may have contributed to greater maize growth in situations where there was a water deficit. Through a variety of mechanisms, bacteria known as plant growth-promoting bacteria (PGPB) can protect plants from abiotic stresses and diseases while also enhancing plant growth. Those bacteria that form close relationships with plants, like endophytes, may be more effective in promoting plant growth. If bacterial inoculants are effective, the use of chemical fertilizers can be curtailed or abolished, which will boost agronomic efficiency by decreasing production costs and pollution to the environment (Souza *et al.*, 2015). Plant growth-promoting bacteria (PGPB) will start to take the place of pesticides in environmental cleanup techniques, horticulture, silviculture, and agriculture (Glick, 2012).

2. MATERIALS AND METHODS

2.1. Sample and Experiment site

First of all ten tiny plants of *Crassula ovata* were taken from nearby nursery (Golden Nursery, Dehradun). Plants were kept in the laboratory for further use.

2.2. Isolation and purification of endophytic bacteria from *Crassula ovata*

For isolation of endophytic bacteria, collected leaves and stem were taken from *Crassula ovata*. Firstly, all plant samples were washed with tap water and then surface sterilization was done with 70% ethanol. All the samples kept into 70% ethanol for 5 min and then with double distilled water repeat this process for 3 times. After that all samples dry onto whattmn filter paper and cut transverse section (TS) of samples under sterile condition and put into two different media nutrient media for isolation of endophytic bacteria, when different colonies were grown. For purification of bacterial culture, we sub culture isolated colonies for three to four times to get purified single isolated colony (Singh *et al.*, 2022, Salo and Novero, 2020).

2.2.1. Scanning electron microscopic analysis of bacterial endophytes

SEM produces images that are greatly magnified by focusing a high energy electron beam on the sample's surface. SEM creates a 3D characterization look that is highly helpful for recognizing the shape and its morphology. To observe the bacterial morphology freshly prepared culture was taken. 1 drop of bacterial sample taken on cover slip, then air dry, after that samples were fixed in 2.5% (v/v) glutaraldehyde for 1 h in 0.1 M PBS and then further in 1% (w/v) osmium tetroxide (OsO₄) for 40 min. The fixed samples were dehydrated using ethanol (20–100%; (v/v) for 2–3 min in each concentration) and then dried in a critical point dryer, and observe under the Scanning Electron Microscope with GEMINI column (ZEISS EVO18 Research, Carl Zeiss Microscopy, GmbH, Jena, Germany) Rakesh *et al.*, 2026.

2.3. Morphological characterization of isolated bacteria strains

The isolated endophytic bacterial strains were characterized by evaluating colony morphology determined by Gram staining. Thin smear was prepared, dry in air and fix by gentle heat. Stain with the crystal violet for 1 min, washed with water and pour Grams iodine for 1 minute. Wash with dd water and decolourizer until no further violet colour drains off then wash with water, lastly counter stain with safranin for about 1 minute and after that wash with dd water, dry and observe under microscope to reveal the bacterial morphology (Mikołajczyk *et al.*, 2023).

2.4. Biochemical testes of isolated bacterial strains from *Crassulaovata*

2.4.1. Urease test

To perform the Urease test, urea broth was prepared and sterilized at 15lbs pressure for 15 min, the medium poured in test tubes. Each tubes were labelled with the bacterial ID of the pure culture isolated sample. A single colony of pure culture was inoculated in each tubes into the centre. Inoculated tubes were inoculated for 24 – 48 hrs at 37±2°C (Balane *et al.*, 2012).

2.4.2. Catalase test

To perform the catalase test of isolated bacterial strains, a 24 hours old bacterial culture was taken on a clean and sterilized glass slide using a sterile tooth pick. 1-2 drop of 3% H₂O₂ was added on slide. Immediate bubble formation indicates positive result for catalase enzyme (Singh *et al.*, 2019).

2.4.3. Motility test

To check the motility of bacterial strains, SIM (Sulphide Indole Motility) medium was prepared and autoclave it. Took a sterile needle, touch a colony on fresh grown culture, Single stab down in the centre of tube about half the depth of tube and incubated at 37± 2°C and then examine the samples (Bhagya *et al.*, 2019).

2.4.4. Nitrate reduction test

Add culture media (Nitrate broth) in a clean test. Inoculate the tube with a heavy inoculum of fresh bacterial culture. Incubate at 35±°C. for 2 hrs. now add 2 drops of reagent A (sulfonic acid) and B (dimethyl-alpha-naphthylamine) and mix well. Observe the red colour develop after 1-2 min to get the positive result.

2.4.5. Starch hydrolysis test

To observe the starch hydrolysing capacity of microbial strains, starch agar plates were prepared. Using a sterile inoculating loop, pick up the sample bacteria from fresh colonies of each strain, streak one line of the samples on plates and incubate at 35± 2°C for at least 48 hrs. After incubation, add few drops of iodine solution over the colonies and observe the formation of clear halo around the colonies (Velmurugan *et al.*, 2021).

2.4.6. H₂S production

SIM (Sulphide Indole Motility) medium was prepared and sterilized at 15lbs pressure for 15 min then medium was poured in the test tubes. Each of the tube was labelled with the bacterial ID of the pure culture isolated from the sample. A single streak was made on each tube for pure culture into the centre of the tube. Inoculated tubes were incubated for 48 hrs at 37±2°C. After incubation black precipitate showed positive H₂S production, while no black precipitate showed negative H₂S production (Bhagya *et al.*, 2019).

2.4.7. Antibiotic sensitivity

Antibiotic sensitivity test was performed using antibiotic impregnated discs (6 mm in diameter). The endophytic bacterial isolates were tested against erythromycin, ampicillin, streptomycin and vancomycin by Kirby Bauer disc-diffusion assay method (Kumar *et al.*, 2016). The quantities of antibiotics used were between 5–30µg/disc. On the basis of inhibition zone recorded,

2.5. Plant growth promoting (PGP) traits analysis

2.5.1. Phosphate solubilisation

To observe the phosphate solubilizing capacity of microbes. Prepare pikovskaya agar media with bromophenol blue dye, then autoclave the media. After that Petri plates were filled with the medium, and allowed to solidify under sterile condition. After that loopful bacterial samples were taken to inoculate the plates. Plates were incubated at 37±2°C for 24hrs. Observed the clear zone around the inoculated area of bacterial sample, Zone of inhibition via dye degradation was recorded for positive result (Kahtaniet *al.*, 2020, Singh *et al.*, 2022, Kumar *et al.*, 2020).

2.5.2. Ammonia production

Freshly grown culture were inoculated in 10ml peptone water in each tube and incubated for 48 – 72hrs at 28 ± 2°C. Nessler reagent was added in each tube. Development of a brown to yellow colour was a positive test for ammonia production (Kahtaniet *al.*, 2020, Panigrahi *et al.*, 2020).

2.5.3. Indole acetic acid production

Preparation of 1% tryptone broth. Dissolve 10g of peptone in one litre of distilled water. Inoculate only tryptone broth with sample. Incubate inoculated and uninoculated tubes at 35±°C for 48 hrs. After 48 hrs of incubation, 1ml of Kovac reagent was added in each tube including control. Shake the tubes gently after interval for 10-15 min. Allow the tubes to stand to permit the reagent to come to the top (Bhagya *et al.*, 2019).

2.5.4. Nitrate reduction test

Add a culture media in a tube. Inoculate the tube with bacterial culture and incubated at 35±°C for 2hrs. Add 2 drops of reagent A (Sulfonic acid) and B (alpha naphthol), now observe development of red colour in 2min (Cipriano *et al.*, 2021).

2.6. Molecular identification of plant growth promoting bacterial endophytes

For the molecular identification of bacterial endophytes. The Qiagen Kit was used to extract high-quality genomic DNA from pure endophytes cultures. The extracted pure DNA was then analyzed by 16S rDNA sequencing using universal bacterial primers with the following sequences: 5'-GGATGAGCCCGCGGCCTA-3' is and 5'-CGGTGTGTACAAGGCCCGG-3' is 16S forward primer and 16S reverse primer, respectively. The reaction volume was 50µl and included a Taq DNA polymerase enzyme 1µl, 10x Taq DNA polymerase assay buffer 10µl, dNTPs 4µl, 16S forward primer 2µl, 16S reverse primer 2µl, isolated bacterial DNA 1µl, nuclease free water 30µl. PCR cycling involved 45 cycles of initial denaturation at 95°C for 3 minutes, denaturation at 94°C for 1 minute, annealing at 50°C for one minute, extension at 72°C for two minutes, and a final extension at 72°C for 7 minutes. Agarose gel electrophoresis was also applied for evaluating the accuracy or purity of the PCR reaction. The PCR results

were then subjected to Sanger sequencing using an ABI Genetic Analyzer 3130xl. The Mega-7 software was utilized for analysis of 16S rDNA genetic sequencing information. Consensus sequences were generated and then compare with the NCBI nucleotide database applying Basic Local Alignment Search Tool (BLAST) algorithm. Species identification was based on minimum 97% sequence similarity with validated reference strains stored in NCBI gene bank database. All validated 16S rRNA gene sequences has been submitted to the NCBI GenBank database, and obtained a unique identification number for isolates (Zoetendaal *et al*, 2006, Rakeshet *et al*, 2026).

3. RESULTS

3.1. Isolation of Endophytic bacteria from explant.

We have isolated nineteen endophytic bacteria from *Crassula ovata*. From these all isolated strains, we have selected five bacterial strains for our further work on the basis of their excellent growth.

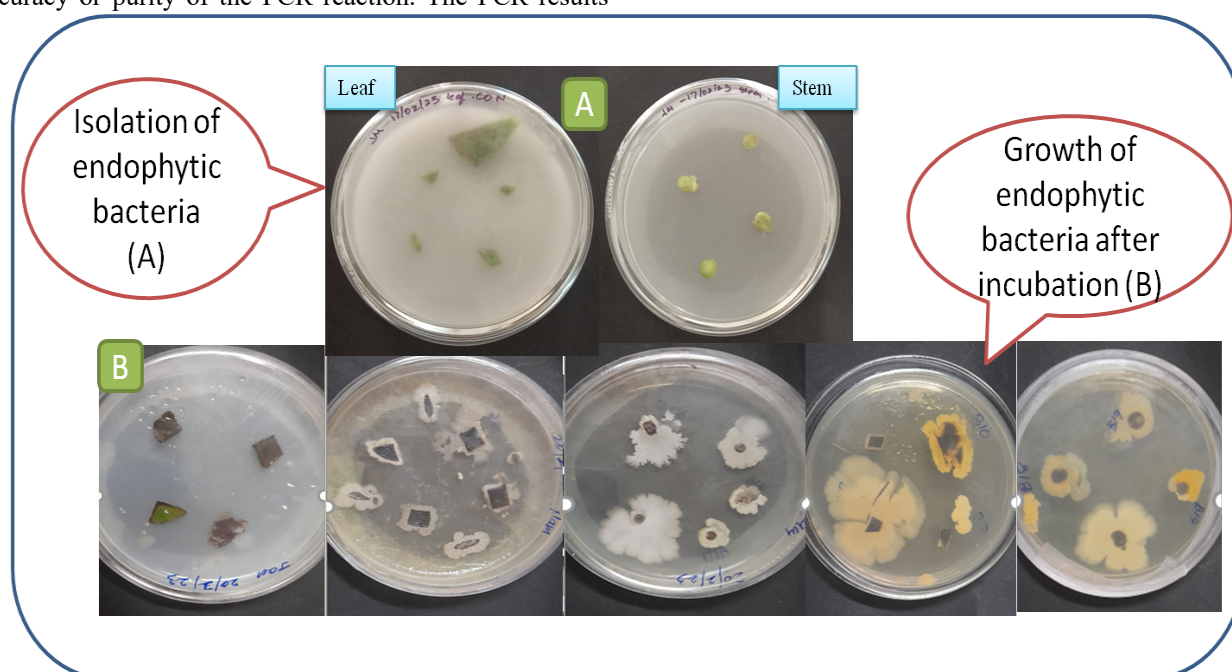


Figure.1. Isolation of endophytic bacteria from leaf and stem of *Crassulaovata*.

After the inoculation of sample on nutrient agar plates, plates were kept in a bacteriological incubator at the temperature $37\pm 2^{\circ}\text{C}$ for 24 hours. When the bacterial colony were observed around the area of cut transverse section of stem, leaves and crushed leaves.

3.1.1. Purification of isolated endophytic bacterial strains and their scanning electron microscopy

Pure cultures were obtained from successive purification of bacterial cultures obtained from *Crassulaovata*. Total nineteen bacterial endophytes were purified, among these, only five strains were taken for further study, all five bacterial strains were also visualized by scanning electron microscopy to check the bacterial morphology, resulted showed in fig. 2

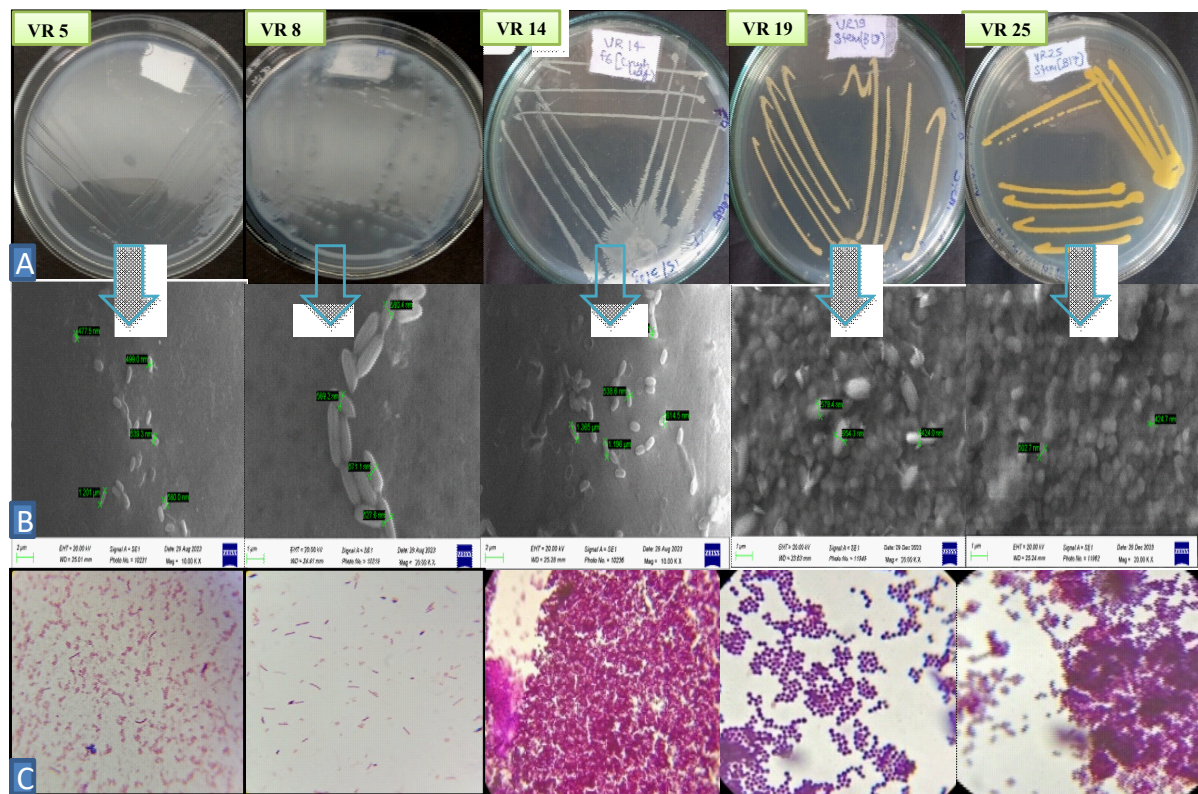


Figure.2. Pure bacterial endophytes of *Crassulaovata*(A), and their scanning electron microscopy, (B) and Gram character (C).

3.2. Morphological identification of isolated bacteria

Isolated bacterial strains were purified by successive streaking, for further test. In this series, all five bacterial isolates were taken for morphological and biochemical characterizations. All strains were gram positive except

VR5, and VR8. Moreover all are spherical shape; the consistency of bacterial colony was mucoid except VR14. The detailed results of morphological characterization are given in Table.1.

Table.1. Morphological characteristics of endophytic bacterial strains isolated from *Crassula ovata*

S.No	Bacterial Strains	Morphological Colony Characteristics				
		Shape	Motility	Colour	Consistency	Gram character
1.	VR 5	Spherical	Partial motile	Colourless	Mucoid	Negative
2.	VR 8	Spherical	Partial motile	Colourless	Mucoid	Negative
3.	VR 14	Spherical	Partial motile	White	Viscid	Positive
4.	VR 19	Spherical	Non-motile	Light yellow	Mucoid	Positive
5.	VR 25	Spherical	Motile	Yellow	Mucoid	Positive

3.3. Biochemical tests of isolated bacterial strains

After the morphological test of the bacterial endophytes, biochemical test was also performed to get the behaviour of endophytes against certain bio-chemicals.

3.3.1. Urease and Catalase test

Five selected bacterial strains were taken for biochemical tests. The urease test was showed positive for all five strains, it indicates that all strains are able to hydrolyze urea by using the enzyme urease. Out of five strains, VR19 showed positive results for catalase whereas VR5, and VR14 showed negative and VR8, VR25 showed partial production of catalase.

3.3.2. Motility test

In the motility tests bacterial strain VR25 was motile, while VR5, VR8, VR14 showed partial results and VR19 showed negative, it indicates that mostly bacteria are motile in nature.

3.3.3. Starch hydrolysis and Nitrate reductase test

These two tests are very important in terms of plant growth and development. In this sequence, VR8, VR14, VR19, completely hydrolyze starch whereas VR5 and VR25 partial hydrolyze starch which means strains have the ability to produce amylase enzyme and utilize starch as a carbon source. Moreover VR8, strains showed positive result in nitrate reductase test, while VR5, VR14, VR19,

VR25 showed negative results. This indicates that all bacterial strains except VR-8 cannot reduce nitrate to nitrite.

3.3.4. H_2S production

In the hydrogen sulphide production test, VR5 showed positive result and VR8, VR14, VR19, VR25 were showed negative result that means one of them have the ability to conversion of Sulphur to Sulphide as H_2S gas, provide as energy source.

Table.2. Biochemical and PGP characteristics of endophytic bacterial strains isolated from *Crassula ovata*

Biochemical tests			Bacterial endophytes				
			VR 5	VR 8	VR 14	VR 19	VR 25
Urease			+	+	+	+	+
Catalase			-	±	-	+	±
Nitrate reductase			-	+	-	-	-
Starch hydrolysis			±	+	+	+	±
H_2S production			+	-	-	-	-
PGP traits analysis							
Phosphate solubilisation	-	-	+		+		+
Ammonia production	+	+	+		+		+
IAA production	±	-	±		-		-

Positive: +, Negative: -, Partial: ±

3.4. Plant growth promoting trait analysis of isolated bacterial strains

Plant growth promoting traits were also evaluated, in this sequence phosphate solubilisation, ammonia production; indole acetic acid production tests were performed. In the phosphate solubilisation test results revealed that VR14, VR19, and VR25 have ability to convert insoluble phosphorus into soluble phosphorous thereby improving

the absorption by plants while VR5 and VR8 do not. Whereas all five strains showed positive result of ammonia production test which showed high efficiency of nitrogen assimilation by the endophytes. In the IAA production test, out of five bacterial strains VR5, VR14 showed partial positive result of IAA production while rest of VR8, VR19, VR25 shows negative results.

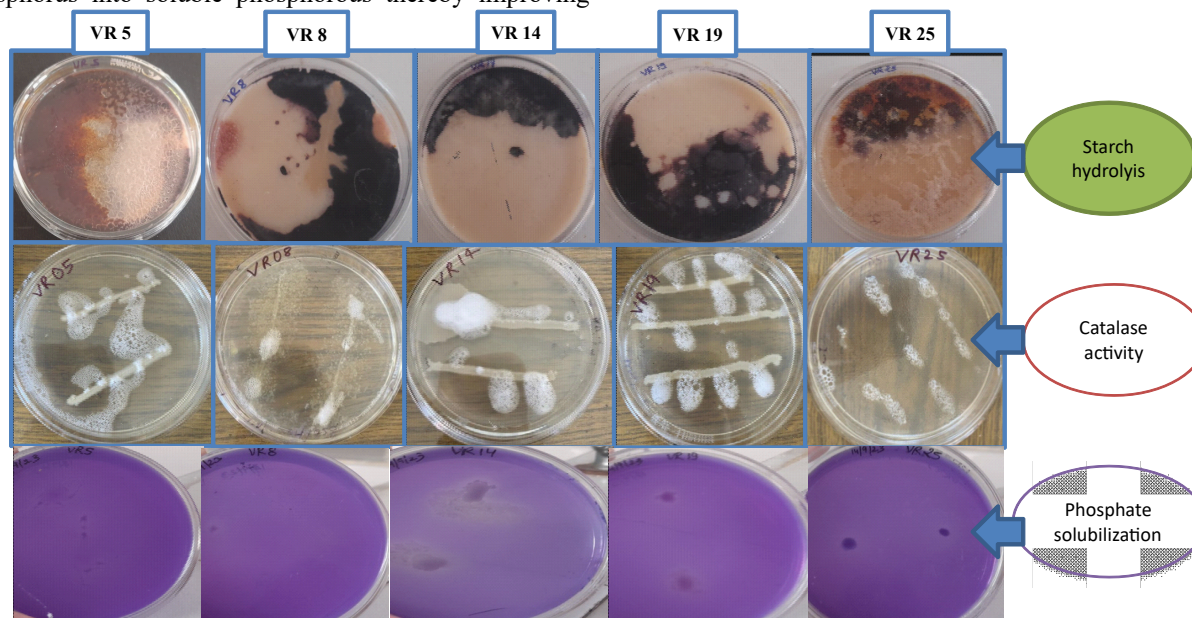


Figure.3. Graphical illustration of plant growth promoting traits of isolated bacterial endophytes

3.5. Antibiotic sensitivity of isolated bacterial endophytes

Antibiotic sensitivity was performed to check the efficacy of antibiotics on *Crassula ovata* bacterial endophytes. Four antibiotics discs were used in the study, results revealed that all four antibiotics showed moderate sensitivity

against bacterial endophytes. Among the all antibiotics streptomycin showed smaller zone of inhibition in VR-5, VR-19 and 25. While all other antibiotics such as erythromycin, ampicillin and vancomycin showed a clear hollow zone of inhibition against all five bacterial endophytes (Figure.4), (Table.3).

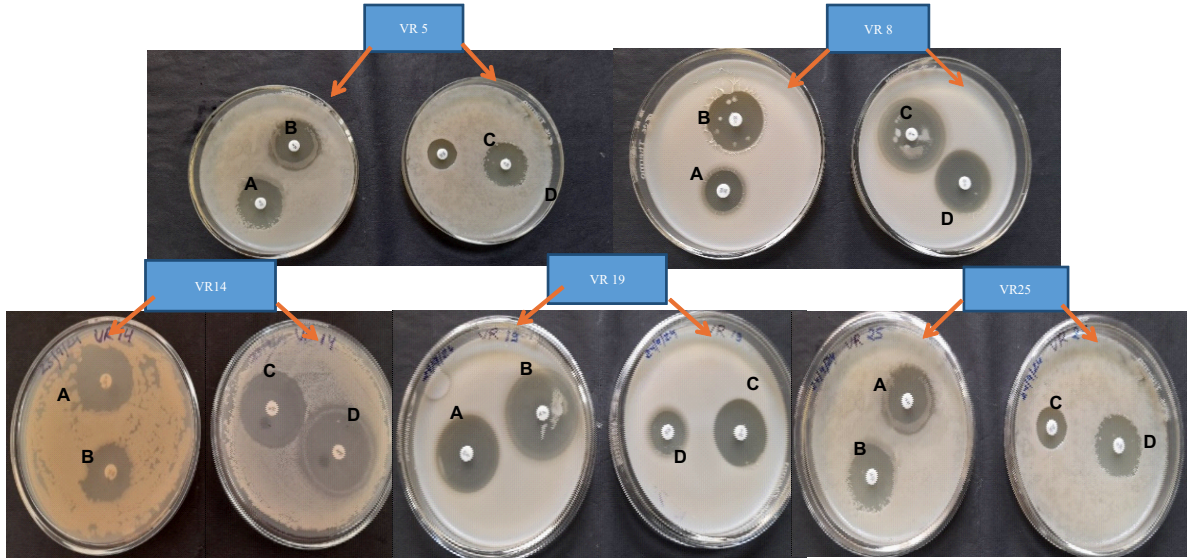


Figure.4. Graphical illustration of Antibiotic sensitivity test of isolated bacterial strains lawn

Table:3. Antibiotic sensitivity test of isolated bacterial strains against few antibiotics

Bacterial Strains	Antibiotics disc (Zone of inhibition in mm diameter)			
	Erythromycin (A)	Ampicillin (B)	Streptomycin (c)	Vancomycin (d)
VR 5	2.3 mm (S)	2.3 mm (S)	1.6 mm (S)	2.4 mm (S)
VR 8	2.3 mm (S)	2.8 mm (S)	2.9 mm (S)	2.8 mm (S)
VR 14	2.8 mm (S)	2.6 mm (S)	2.7 mm (S)	2.9 mm (S)
VR 19	2.9 mm (S)	3.1 mm (S)	1.8 mm (S)	2.6 mm (S)
VR 25	2.4 mm (S)	2.4 mm (S)	1.3 mm (S)	2.8 mm (S)

R: Resistant; S: Susceptible

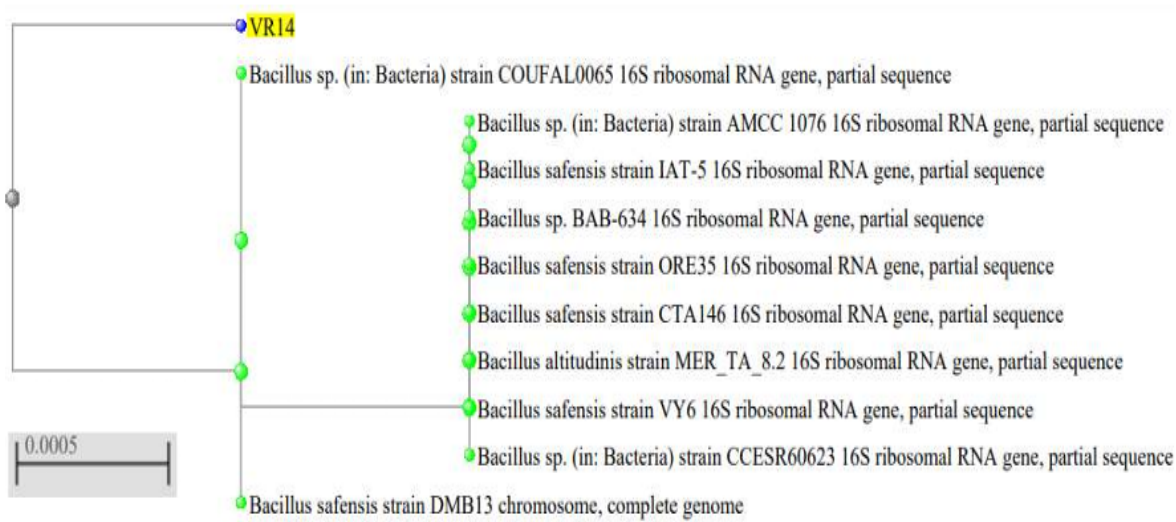
4. MOLECULAR CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA FROM CRASSULA OVATA

On the basis of excellent growth and having plant growth promoting trait analysis only two strains (VR 14 and

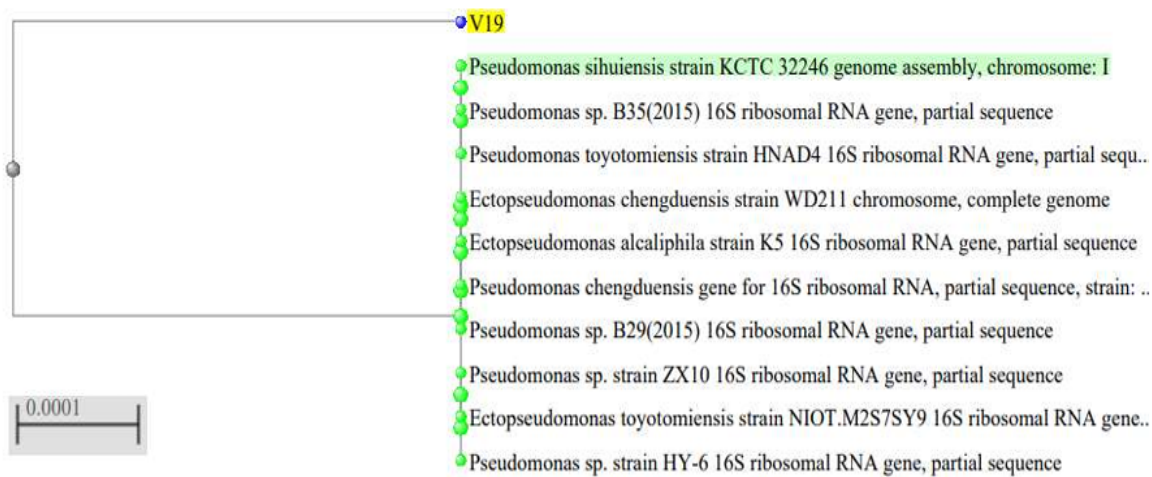
VR19) were taken for the molecular identification using 16S rRNA gene sequencing method. Results revealed that VR14 has 99.8% homology with Bacillus genus, while VR19 has 99.75% homology with Pseudomonas sichuanensis strain Table.4.

Table.4.Endophytic bacterial strains VR14 and VR19 of Crassulaovatawith accession no. and nearest phylogenic strains

S.No.	Isolated strains	Accession no. Obtained from NCBI	Nearest phylogenic strain	Similarity %
1.	VR 14	PV012479	Bacillus	99.85%
2	VR 19	PV012480	Pseudomonas sichuanensis	99.78%



Sl. No.	Organism Name	Accession No.	% Match
1	Bacillus sp. (in: Bacteria) strain COUFAL0065 16S ribosomal RNA gene.	MN046307.1	99.85%
2	Bacillus safensis strain DMB13 chromosome.	CP150833.1	99.85%
3	Bacillus sp. (in: Bacteria) strain AMCC 1076 16S ribosomal RNA gene.	MN826416.1	99.77%
4	Bacillus safensis strain IAT-5 16S ribosomal RNA gene.	OR815960.1	99.77%
5	Bacillus sp. BAB-634 16S ribosomal RNA gene.	KF913651.1	99.77%
6	Bacillus safensis strain ORE35 16S ribosomal RNA gene.	PQ182253.1	99.77%
7	Bacillus safensis strain CTA146 16S ribosomal RNA gene.	MW700038.1	99.77%
8	Bacillus altitudinis strain MER_TA_8.2 16S ribosomal RNA gene.	KT719418.1	99.77%
9	Bacillus safensis strain VY6 16S ribosomal RNA gene.	OQ132858.1	99.77%
10	Bacillus sp. (in: Bacteria) strain CCESR60623 16S ribosomal RNA gene.	MW742483.1	99.77%



Sl. No.	Organism Name	Accession No.	% Match
1	<i>Pseudomonas sihuiensis</i> strain KCTC 32246 genome assembly.	LT629797.1	99.78%
2	<i>Pseudomonas</i> sp. B35(2015) 16S ribosomal RNA gene.	KT380538.1	99.78%
3	<i>Pseudomonas toyotomiensis</i> strain HNAD4 16S ribosomal RNA gene.	ON115181.1	99.78%
4	<i>Pseudomonas</i> sp. strain ZX10 16S ribosomal RNA gene.	MG760368.1	99.78%
5	<i>Ectopseudomonas toyotomiensis</i> strain NIOT.M2S7SY9 16S ribosomal RNA gene.	PP587696.1	99.78%
6	<i>Pseudomonas</i> sp. strain HY-6 16S ribosomal RNA gene.	MK355517.1	99.78%
7	<i>Ectopseudomonas chengduensis</i> strain WD211 chromosome.	CP129400.1	99.78%
8	<i>Ectopseudomonas alcaliphila</i> strain K5 16S ribosomal RNA gene.	KY928092.1	99.78%
9	<i>Pseudomonas chengduensis</i> gene for 16S ribosomal RNA., strain: AF100	LC015559.1	99.78%
10	<i>Pseudomonas</i> sp. B29(2015) 16S ribosomal RNA gene.	KT380533.1	99.78%

Figure.4.Phylogenic tree of identified strains from *Crassulao vata* and their BLAST analysis.

5. DISCUSSION

The *Crassula ovata* plants are rich in medicinal compounds of human interest. Molecular and genetic studies of medicinal plants bring up a wide range of new research opportunities. Then, there was plenty of need for more research on *Crassula ovata*. The present study was concern with endophytic bacteria isolated from stem and leaf of Jade plant. Out of 19 bacteria we have selected 5 strains according to their growth and morphology. Out of five, two bacterial strains were identified by 16s RNA gene sequencing. VR14 has 99.85% similarity with *Bacillus* and VR19 has 99.75%, similarity with *Pseudomonas sichuanensis*. In this study authors explored the biochemical and PGP activities of bacterial strains. During the biochemical activities all 5 strains showed production of urease. Whereas VR19 shows catalase, VR25 showed motility, VR5 showed nitrate reduction, VR8, 14, 19 showed starch hydrolysis tests, and VR5 showed H₂S production. Moreover in PGP trait analysis VR14, 19 and 25 showed phosphate solubilisation, whereas all five strains showed ammonia production. In another study by Duhan *et. al* in 2020, also reported some identified endophytes from leaf and stem of *T. Cardifolia* plant. These endophytes have potential to synthesize bioactive compounds and showed antifungal and antibacterial activities. In this sequence Kumar *et. al.*, 2016 stated that six endophytic bacteria isolated from *Curcuma longa* L. showed almost positive result in IAA, phosphate solubilisation and it also utilized nitrogen and carbon source. All six strains showed antibiotic properties against *E.coli* and *Enterobacter*. Here in our study the bacterial endophytes were isolated from jade plant, that is a succulent. In another study Singh *al.*, 2019, also identified some temperature tolerant bacterial endophytes from tomato. Kahtani *et. al*, 2022 also demonstrated endophytic bacterial strains from *Fagonia mollis* and *Achillea fragrantissima* had plant growth promoting activity and also showed phosphate solubilizing activity which enhance plant capacity to absorb nutrient and tolerate stress and

also support the growth and productivity of plants. Several other studies on endophytes bacteria isolated from *Gloriosa superba* and *Momordica charantia* have excellent PGP activity when inoculated with the crops (Kiros *et. al*, 2023; Singh *et al.*, 2022). Out of 13 endophytic bacteria from *Anredera cordifolia*, one *P. aeruginosa* showed antimicrobial activity and have higher secondary metabolites, which is a source of pharmacological active compounds (Nxumalo *et al.*, 2020). In this article Mikołajczyk *et al.*, 2023 said that endophytic bacteria isolated from *Urtica dioica* L. showed variety of secondary metabolites and produced biological activity and also it has polyphenols which showed antioxidant, anti-inflammatory and ant cancerous activity.. In another study endophytic bacterial isolates from *Solanum lycopersicum* L. have some medicinal and pharmaceutical properties. Inoculation of the endophytic bacterial strains into tomato plants enhanced its bioactive compounds like flavonoid and lycopene (Singh *et. al*, 2021).

6. CONCLUSION

In this study, bacterial endophytes are isolated from the succulent plant *Crassula ovata*. All nineteen bacterial endophytes were purified and on the basis of excellent growth, five were taken for morphological, biochemical and plant growth promoting trait analysis. Out of five strains, only two strains were selected for molecular identification, those are having excellent PGP traits. Those two bacterial endophytes viz; VR14 and VR19 belong to the genera *Bacillus* and *Pseudomonas* respectively. This research study concluded that, these two strains having capacity to promote the growth and development of succulent medicinal plants such as *Crassula ovata*. In the near future the microbial endophytes, VR 14 and VR19 might be used as microbial biofertilizer for the plant growth promotion of useful crops which are mostly drought resistant.

ACKNOWLEDGEMENT

The authors sincerely thank the Department of Biotechnology, SALS and Division of Research and Innovation, Uttaranchal University, Dehradun for providing necessary facility for the completion of my Ph.D work

Conflicts of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Funding source

Not applicable

Author contributions

All research work has been done by VR; MS, wrote the manuscript. MS supervised the study; MS reviewed the manuscript.

Data availability

The raw sequence data presented in the study are deposited in the National Centre for Biotechnology Information advances science and health (NCBI), accession number PV012479, PV012480.

REFERENCES

- Ahmed ABA, Taha RM (2014). Indirect Regeneration and Somatic Embryogenesis from Leaf and Stem Explants of *Crassula ovata* (Mill.) Druce—An Ornamental Medicinal Plant. *Int. J. Biomed. Biol. Eng.* **8**(12), 1310-1313.
- Balan SS, Fathima F, Jayalakshmi S (2012) Characterization of urease enzyme from marine bacterium *Klebsiella* species. *Afr. J. Microbiol. Res.* **6**(30), 5914-5923, <https://doi.org/10.5897/AJMR12.218>
- Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol. Ecol.* **51**(2), 215-229, <https://doi.org/10.1016/j.femsec.2004.08.006>
- Bhagya M, Nagaraju K, Biradar BJP, Santosh GP, Gundappagol RC (2019) Isolation and Characterization of endophytic bacteria from nodule, root and seeds of green gram (*Vignaradiata* L.), *Int. J. Pure Appl. Biosci.* **7**(4), 319-328, DOI: <http://dx.doi.org/10.18782/2320-7051.7737>
- Brooks DS, Gonzalez CF, Appel DN, Filer TH. (1994) Evaluation of endophytic bacteria as potential biological-control agents for Oak Wilt, Biological control, **4**(4), 373-381, <https://doi.org/10.1006/bcon.1994.1047>
- Chokhone, K., Talukdar, N., Sarma, MP., Das, K., Kalita, PP (2017) Screening of phytochemicals and evaluation of anti-microbial, anti-oxidant and in-vitro antidiabetic activity of *Crassula ovata* leaves, *Int. J. Pharm. Sci. Res.* **8**(2), 859. [http://doi.org/10.13040/IJPSR.0975-8232.8\(2\).859-64](http://doi.org/10.13040/IJPSR.0975-8232.8(2).859-64)
- Cipriano MAP, Iorio RDPF, Dimitrov MR, Andrade SALD, Kuramae EE and Silverira APDD (2021) Plant-growth endophytic bacteria improve nutrient use efficiency and modulate foliar N-metabolites in sugarcane seedling, *Microorganisms*, **9**(3): 479. <http://doi.org/10.3390/microorganisms9030479>
- Duhan P, Bansal P, and Rani S (2020) Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinosporacordifolia*. *S. Afr. J. Bot.* **134**, 43-49. <https://doi.org/10.1016/j.sajb.2020.01.047>.
- Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications, *Scientifica*, Article ID 963401, <https://doi.org/10.6064/2012/963401>
- Hassan MHA, Elwekeel A, Moawad A, Afifi N, Amin E, Amir ELD (2021) Phytochemical constituents and biological activity of selected genera of family *Crassulaceae*: A review. *S. Afr. J. Bot.* **141**, 383-404, <https://doi.org/10.1016/j.sajb.2021.05.016>
- Kahtani MDFAL. Fouda A, Attia, KA, Otaibi FA, Eid AM, Ewais EED, Hijri M, Arnaud MS, Hassan SED, Khan N, Hafez YM, and Abdlaal KAA (2020) Isolation and characterization of plant growth promoting endophytic bacteria from Desert plants and their application as bioinoculants for sustainable agriculture. *Agronomy*, **10**(9), 1325; <https://doi.org/10.3390/agronomy10091325>.
- Kiros T, Ebu SM, Melaku Y, Tesfa T, and Dekebo A (2023) Isolation and identification of endophytic bacteria and associated compound from *Gloriosasuperba* and their antibacterial activities. *Heliyon*. **9**, e22104. <https://doi.org/10.1016/j.heliyon.2023.e22104>.
- Kumar V, Jain L, Jain SK, Chaturvedi S, and Kaushal P (2020) Bacterial endophytes of rice (*Oryzasativa* L.) and their potential for plant growth promotion and antagonistic activities, *S. Afr. J. Bot.* **134**, 50-63. <https://doi.org/10.1016/j.sajb.2020.02.017>
- Kumar A, Singh R, Yadav A, Giri DD, Singh PK, and Pandey KD (2016) Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech*, **6**(1):60. <https://doi.org/10.1007/s13205-016-0393-y>.
- Mikołajczyk OM, Chlebicz M, Kawecka M, Michalak A, Prucnal F, Nielipinski M, Filipek J, Jankowska M, Perek Z, Drozdzyński P, Rutkowska N, and Otlewska, A (2023) Endophytic bacteria isolated from *Urtica dioica* L.- preliminary screening for enzyme and polyphenols production, *Microbial Cell Factories*, **22**, 169. <https://doi.org/10.1186/s12934-023-02167-2>
- Muiruri MD, and Mwangi W (2016) Phytochemical and antimicrobial activity of (*Crassula ovata*) Jade

- plant* on different strains of bacteria, *Eur. J. Med. Plants*, **11**(1), 1-12, <https://doi.org/10.9734/EJMP/2016/19753>
17. Nxumalo CI, Ngidi LS, Shandu JSE, Maliehe TS (2020) Isolation of endophytic bacteria from the leaves of *Anrederacordifolia* CIX1 for metabolites and their biological activities. *BMC Complement. Med. Ther.* **20**:300, <https://doi.org/10.1186/s12906-020-03095-z>.
18. Ortu G, Bertetti D, Gullino ML, Garibaldi A (2013) A new forma specialism of *Fusarium oxysporum* on *Crassula ovata*. *J. Plant Pathol.* **95**(1), 33-39, <https://www.jstor.org/stable/23721733>
19. Oshnoei FT, Harighi B, and Abdollahzadeh J (2017) Isolation and identification of endophytic bacteria with plant growth promoting and biocontrol potential from oak trees, *For. Pathol.* **47**(5), e12360, <https://doi.org/10.1111/efp.12360>.
20. Panigrahi S, Mohanty S, Rath CC (2020) Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with indole acetic acid (IAA) production and plant growth promoting capabilities against selected crops, *S. Afr. J. Bot.* **134**, 17-24. <https://doi.org/10.1016/j.sajb.2019.09.017>
21. Pereira SIA, Abreu D, Moreira H, Vega A, Castro PML (2020) Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions. *Heliyon*, **6**, 10, e05106. <https://doi.org/10.1016/j.heliyon.2020.e05106>
22. Rakesh, Munjal SD, Sharma N, and Singh M (2026) A comprehensive analysis of gut microbial profiles and their clinical implications in Uttarakhand population. *J. Exp. Zool. India*, **29**, 1325-1336. <https://doi.org/10.51470/jez.2026.29.2.1325>
23. Rijavec T, Lapanje A, Dermastia M, Rupnik M (2007) Isolation of bacterial endophytes from germinated maize kernels. *Can. J. Microbiol.* **53**(6), 802-808, <https://doi.org/10.1139/W07-048>.
24. Salo EN, and Novero A (2020) Identification and characterisation of endophytic bacteria from Coconut (*Cocos nucifera*) Tissue Culture, *Trop. Life Sci. Res.* **31**(1): 57-68, <https://doi.org/10.21315/tlsr2020.31.1.4>
25. Singh M, and Pandey KD (2021) Endophytic bacterial strains modulated synthesis of lycopene and bioactive compounds in *Solanum lycopersicum* L. fruit. *Biocatal. Agric. Biotechnol.* **35**, 102088. <https://doi.org/10.1016/j.bcab.2021.102088>
26. Singh M, Kumar A, Pandey KD (2019) Biochemical and molecular identification of *Solanum lycopersicum* L. temperature tolerant bacterial endophytes. *Biocatal. Agric. Biotechnol.* **22**, 101409. <https://doi.org/10.1016/j.bcab.2019.101409>.
27. Singh M, Kumar A, Singh R, Pandey KD (2017). Endophytic bacteria: a new source of bioactive compounds. *3 Biotech*, **7**:315. <https://doi.org/10.1007/s13205-017-0942-z>.
28. Singh R, Pandey KD, Singh M, Singh SK, Hashem A, Arjani ABFA, Allah, EFA, Singh PK, and Kumar A (2022) Isolation and characterization of endophytes bacterial strains of *Momordica charantia* L. and their possible approach in stress management. *Microorganisms*, **10**(2): 290, <https://doi.org/10.3390/microorganisms10020290>
29. Souza RD, Ambrosini A, and Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils, *Genetics and Mol Biol.* **38**(4): 401-419, <https://doi.org/10.1590/S1415-475738420150053>
30. Stone JK (2000) An overview of endophytes microbes: endophytism defined. In: C. D Bacon and JF White. (eds.), Taylor and Francis, *Microbial endophytes*, pp, 3-29. Sturz AV, Christie BR, and Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.* **19**(1), 1-30, <https://doi.org/10.1080/07352680091139169>
31. Tan HM, Cao LX, He ZF, Su GJ, Lin B, and Zhou SN (2006). Isolation of endophytic actinomycetes from different cultivars of tomato and their activities against *Ralstonia solanacearum* in vitro. *World J. Microbiol. Biotechnol.* **22**, 1275-1280, <https://doi.org/10.1007/s11274-006-9172-y>
32. Velmurugan S, Anokhe A, and Kalia V (2021) Biochemical characterisation of starch hydrolysing bacteria, *AgriCos e-newsletter*, **2**, 11, 19, ISSN:2582-7049.
33. Zoetendal EG, Vaughan EE, Vos WMD (2006) A microbial world within us. *Mol Microbiol.* **59**(6):1639-50. <https://doi.org/10.1111/j.1365-2958.2006.05056.x>.